New Insights Into Autophagy Dysfunction Related to Amyloid Beta Toxicity and Neuropathology in Alzheimer's Disease

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Abstract

The fine control of neuronal proteostasis is an essential element that preserves cell viability. Advancing age is a major risk factor for Alzheimer's disease (AD), and autophagy is thought to dictate normal and pathological aging through intricate molecular machinery controlling protein aggregation. Although the role of autophagy dysfunction in AD is known, the dynamic changes during the progression of the disease remain unclear. Recent studies have provided new insight into the molecular mechanisms that link defective autophagy and cellular fate, underscoring the pathogenic events associated with AD. Here, we will focus on recent studies that underpin a distinct role for autophagy deficits and highly localized autophagic defects, impacting primarily the amyloidogenic pathway activity. By uniquely assessing the dynamic changes in key proteins during the disease progression in the context of the autophagy machinery function and amyloid beta toxicity, specifically, a connect between protein degradation failure and cell death susceptibility is revealed which may suggest new avenues for the development of better targeted therapeutic interventions.

International Review of Cell and Molecular Biology
ISSN 1937-6448
http://dx.doi.org/10.1016/bs.ircmb.2017.07.002

1. INTRODUCTION

Alzheimer's disease (AD) is the most common neurodegenerative disease and a leading cause of dementia in the elderly (Andrieu et al., 2015). The confirmatory AD diagnosis is based on two major histopathologic hallmarks: senile plaques, which are extracellular deposits of amyloid beta (Aβ) peptides, and intraneuronal neurofibrillary tangles (NFTs), which are somatic inclusions of hyperphosphorylated, microtubule-associated protein tau (Mattson, 2004). With advancing age being the most prominent risk factor for the disease, and ~44 million people currently affected worldwide, the prevalence of AD is predicted to reach epidemic proportions by 2050. AD is clinically characterized by a gradual loss of cognitive function, ultimately leading to a loss of autonomy, thus necessitating full-time medical care (Prince et al., 2013). Although intensive efforts have been directed toward developing AD disease-modifying therapies, current treatment strategies ameliorate disease symptoms alone (Anand et al., 2014; Di Santo et al., 2013). In the continued absence of effective therapeutic strategies to either delay or deflect disease progression, AD is set to pose an enormous burden on society and public health systems.

Several hypotheses have been put forward to better understand the multifactorial pathophysiology of the disease, including the amyloid cascade hypothesis (ACH), the cholinergic and tau hypothesis, as well as inflammation (Kurz and Perneczky, 2011); however, many molecular aspects and their dynamic changes during disease progression remain unclear. The ACH, the most widely accepted mechanistic hypothesis for AD, posits that an imbalance between the production and clearance of Aβ peptides is a very early, often initiating factor in disease onset (Hardy, 2009; Hardy and Higgins, 1992). Although extracellular Aβ plaques and intraneuronal NFTs are defining hallmarks of AD neuropathology, a growing body of literature suggests that deficits in the autophagy–lysosomal pathway are likely to precede the formation of these pathological hallmarks (Nixon and Yang, 2011; Zare-Shahabadi et al., 2015). Neurons are postmitotic cells, with high demands for ATP and protein synthesis processes, to maintain specialized structural integrity for transport processes and intercellular communication. Therefore, neuronal integrity is more sensitive to alterations in basal autophagy than any other cell types (Nikoletopoulou et al., 2015). The autophagy–lysosomal pathway represents the main degradative system in neurons and is extremely important in maintaining cellular homeostasis, which requires the continuous

turnover of aberrant proteins and dysfunctional organelles (Shehata et al., 2012). Although basal autophagy exists in all eukaryotic cells, governing the removal of primarily long-lived and naturally occurring misfolded proteins (Mizushima et al., 2008), a landmark study using a green fluorescent protein-tagged microtubule-associated protein 1A/1B-light chain 3 (GFP-LC3) transgenic mouse model demonstrated that autophagy is distinctly regulated in neurons (Mizushima et al., 2004). Significant differences in basal autophagy between neuronal and nonneuronal cells are evident (Mitra et al., 2009) and pioneering loss-of-function studies further support an especially critical role for basal autophagy in the brain (Hara et al., 2006; Komatsu et al., 2006, 2007). For instance, tissue-specific deletion of key autophagy genes in murine muscle, heart or central nervous system result in muscular atrophy, cardiac hypertrophy and neurodegeneration, respectively (Hara et al., 2006; Nair and Klionsky, 2011; Nakai et al., 2007). Additionally, knockout of an essential autophagy gene, ATG5, in mice, causes lethality soon after birth, suggesting a crucial role for autophagy during the early neonatal period in the maintenance of energy homeostasis (Kuma et al., 2004). More recently, a systems biology study highlighted the pivotal role of dysregulated autophagy in neurodegenerative diseases, where toxic protein aggregates and damaged organelles accumulate within specific types of neurons and lead to neuronal dysfunction and ultimately, demise (Caberlotto and Nguyen, 2014). Advancing age is a major risk factor for AD, and autophagy is thought to dictate normal and pathological aging through intricate molecular machinery controlling neuronal intracytoplasmic levels of toxic protein aggregates (Rubinsztein et al., 2011).

Recent studies have provided new insights into the molecular mechanisms that underlie defective autophagy and aging, which may underscore the pathogenic events associated with neurodegenerative diseases. In addition, a number of recent developments focusing on molecular tools that measure the rate of protein degradation through autophagy, i.e., autophagic flux, have been discovered, suggesting new ideas for linking proteostasis and the loss thereof with cell death onset. Therefore, this review will focus on recent studies that underpin a distinct role for autophagy deficits in AD, and the identification of precisely localized autophagic defects. By uniquely assessing the dynamic changes in key proteins during disease progression, from mild cognitive impairment to late stage AD, in the context of autophagic function and amyloidogenesis, a connect between protein degradation failure and cell death susceptibility is revealed which may drive the development of better targeted therapeutic interventions.

2. AUTOPHAGY AND ITS MACHINERY

Autophagy is a tightly regulated pathway (Mizushima et al., 2008) and its machinery is induced by a variety of signals including nutrient starvation, hypoxia, and ER stress (Dohi et al., 2012; Nijholt et al., 2011; Son et al., 2012b; Young et al., 2009). Three distinct classes of autophagy exist, depending on the route through which the sequestered cargo is delivered to the lysosomal compartment for digestion: microautophagy, chaperone-mediated autophagy (CMA), and macroautophagy (Boya et al., 2013). In microautophagy, invaginations of the lysosomal membrane directly engulf portions of the cytoplasm. CMA involves the selective recognition and unfolding of substrate proteins bearing a KFERQ amino acid motif by a complex of cytosolic chaperones. These substrates bind to the CMA-specific lysosomal protein LAMP-2A and are translocated across the lysosomal membrane for degradation (Kaushik and Cuervo, 2012). Macroautophagy is the major type of autophagic pathway and differs from the other two types in that a flat membraned cistern known as a phagophore, that forms de novo, sequesters the substrates. The phagophore elongates and seals to surround the cytoplasmic material, forming a double membraned vesicle called an autophagosome, which then fuses with endosomal and lysosomal vesicles, in which the hydrolytic degradation of sequestered contents occurs (Fig. 1) (He and Klionsky, 2009; Ravikumar et al., 2010b). This process provides enormous dynamic adaptation, whereby in addition to cytosolic proteins, selective aggregated proteins and damaged organelles, such as mitochondria, peroxisomes, and invasive microbes are targeted (Johansen and Lamark, 2011; Komatsu and Ichimura, 2010; Wong et al., 2008). We will focus our discussion on macroautophagy, herein referred to as autophagy, as this pathway specifically, is highly and distinctively impaired in AD (Boland et al., 2008; Nixon et al., 2005; Yu et al., 2005).

Identification of several autophagy-related genes (ATGs), i.e., ATG1-ATG35, whose functions are thought to be conserved from yeast to mammals (Meijer et al., 2007), has greatly aided the dissection of the autophagic pathway. Mechanistically, autophagy can be categorized into the following essential steps: initiation, expansion of the autophagosome membrane, maturation of the autophagosomes, and degradation. Each step requires tight regulation of several, indispensable, ATG genes (Fig. 1) (Feng et al., 2014; Maday and Holzbaur, 2014; Parzych and Klionsky, 2014).

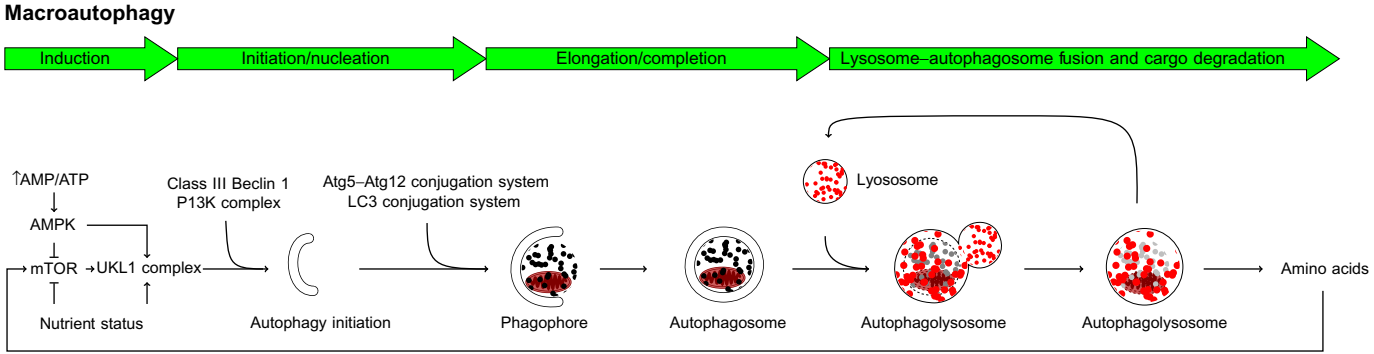


Fig. 1 The multistep pathway of autophagy forming an energetic feedback loop from energetic sensing to cargo degradation and amino acid release. The rate of flow through this pathway is termed autophagic flux and contributes to proteostasis, quality control, and metabolite substrate generation.

Although the initiation of autophagy is constitutively kept at low levels, diverse environmental stressors can act as inducers of this degradative machinery (Mizushima et al., 2008). Starvation is the classic stimulus for autophagy induction, allowing cells to respond to nutrient scarcity through increased allocation of amino acids from proteins sequestered as autophagic cargo (Hosokawa et al., 2009). The classical pathway regulating mammalian autophagy involves the serine/threonine kinase, mammalian target of rapamycin (mTOR), in which autophagy is negatively regulated by the mTOR complex 1 (mTORC1) (Guertin and Sabatini, 2009; Noda and Ohsumi, 1998; Ravikumar et al., 2010b). Briefly, during conditions of nutrient availability, active mTORC1 phosphorylates ULK1, which is sequestered into a complex with the autophagic protein, Atg13, and focal adhesion kinase family interacting protein of 200 kDa (FIP200), thereby inhibiting autophagy. In the absence of nutrients, amino acid deprivation, growth factor withdrawal, or treatment with rapamycin, ULK1 phosphorylation by mTORC1 is reduced and the ULK1 complex is activated through auto-phosphorylation, which in turns phosphorylates Atg13 and FIP200. Activated ULK1 phosphorylates and activates the Beclin1-vacuolar protein sorting 34 (VPS34) complex, which contains Beclin1, VPS34, and Atg14L (Itakura et al., 2008). The activated Beclin1 complex functions as a class III phosphatidylinositol 3-kinase (PI3KCIII), to produce phosphatidylinositol 3-phosphate (PI3P), which in turn provides a platform to recruit PI3P-binding proteins during the nucleation process (Hosokawa et al., 2009; Kim et al., 2011; Sarkar, 2013). Atg14L targets the Beclin1/PI3KCIII complex to the specialized subdomain of the endoplasmic reticulum (ER), called the omegasome, which consequently triggers phagophore formation (Axe et al., 2008; Matsunaga et al., 2010). Although the Golgi apparatus, mitochondria and plasma membrane may also act as membrane sources for phagophore formation under certain conditions (Axe et al., 2008; Ravikumar et al., 2010a), a growing number of studies have revealed that phagophores locate between two cisterns of rough ER in starved cells (Hayashi-Nishino et al., 2009; Ylä-Anttila et al., 2009). In support of this, the omegasome marker protein DFCP1 was found to be localized in delicate membrane tubules extending from the ER, thus making the ER the most likely membrane source for the formation of phagophores (Uemura et al., 2014). Beclin1 has many binding partners such as Atg14L, UV-irradiation-resistance-associated gene (UVRAG), and Bcl2 (He and Levine, 2010).

Positive regulators of Beclin1 function and autophagy include AMBRA1, UVRAG and Bif-1; and negative regulators include the anti-apoptotic proteins Bcl2 and BCL-XL (Pattingre et al., 2005; Sinha and Levine, 2008). Bcl2 inhibits autophagy indirectly through its interaction with Beclin1 (Pattingre et al., 2005). This Bcl2 modulation aims to prevent excessive degradative activity of the cells and subsequent cell death (Pattingre et al., 2005). Positive UVRAG association with Beclin1 has been shown to enhance PI3KCIII activity and induces autophagosome formation (Liang et al., 2006). In addition to this, Beclin1 is also involved in various pathophysiological processes associated with neurodegeneration (He and Levine, 2010). Its functional role within the PI3KCIII complex is especially important for cellular homeostasis, as it maintains the dynamic coordination of specific endosomal trafficking and autophagy steps (e.g., initiation and nucleation) (Liang et al., 2008; Matsunaga et al., 2009; Russell et al., 2013). For example, Beclin1 has also been found to restore autophagic activity in ATG6-disrupted yeast, becoming one of the first identified genes to positively regulate autophagy (Liang et al., 1999).

Two ubiquitin-like conjugation systems are involved in the elongation and expansion of the phagophore membrane. Firstly, Atg12 is conjugated to Atg12–Atg5, then interacts with Atg16L, which is necessary for autophagosome formation (Mizushima et al., 2003). This is followed by the conjugation of LC3 to the lipid phosphatidylethanolamine (PE). LC3 is cleaved at its C-terminus by Atg4 to form the cytosolic LC3-I, which is conjugated with PE to generate LC3-II (Tanida et al., 2004). LC3-II specifically associates with autophagosomes and LC3-based assays are commonly used to measure autophagy (Tanida et al., 2002). Although LC3 levels correlate with autophagosome numbers, it is critical to monitor the rate of protein degradation through autophagy, i.e., autophagic flux, through the entire pathway (Klionsky et al., 2016; Loos et al., 2014). Following formation, autophagosomes undergo a maturation process that includes fusion with endosomal and lysosomal vesicles (Eskelinen, 2005). Studies show that undisturbed autophagic flux requires a tight cooperation between the endosomal compartments, including early- and late endosomal sorting/maturation, and autophagosomes (Fig. 2) (Eskelinen, 2005). For instance, before fusing with lysosomes to form autolysosomes, autophagosomes can also directly fuse with early- and/or late endosomes, to form hybrid structures called amphisomes, which then fuse with lysosomes, thereby forming autolysosomes in which the cytosolic content is degraded (Fig. 2) (Berg et al., 1998; Filimonenko et al., 2007; Liou et al., 1997).

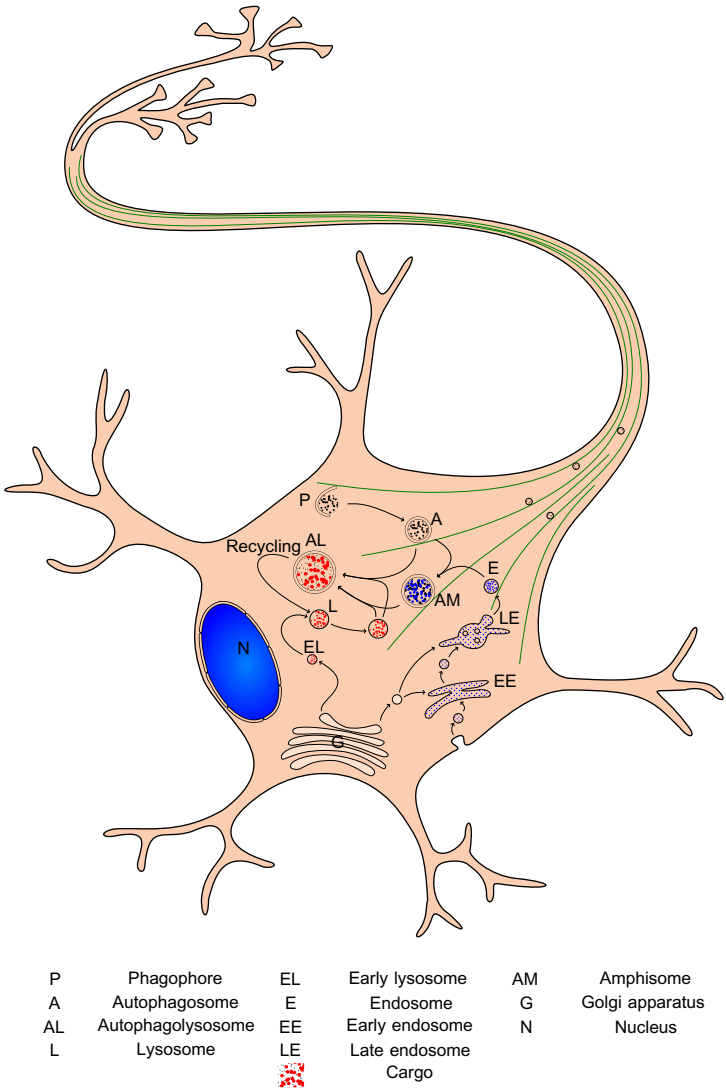


Fig. 2 Functional neuronal autophagy. High autophagy flux is typical for neurons and requires adequate autophagosome synthesis and degradation to maintain steady state. Endosome and autophagosome transport as well as autophagosomal/lysosomal fusion contributes to the successful cargo delivery, preventing the buildup of protein aggregates.

3. Aβ BIOGENESIS AND AD-RELATED TOXICITY

Amyloid precursor protein (APP), an evolutionary conserved type 1 transmembrane protein, is unequivocally linked to AD pathogenesis as the unique source of neurotoxic forms of Aβ (Chen, 2015; Rajendran and Annaert, 2012). During early development, APP is highly enriched at the growth cones of developing neurites (Ramaker et al., 2016; Sabo et al., 2003). In more mature neurons, APP localizes to focal adhesion sites and within pre- and postsynaptic structures of the central and peripheral nervous tissue, suggesting a functional role in neuritic growth and synaptic plasticity (Ashley et al., 2005; Yamazaki et al., 1997). APP is synthesized in the ER and transported to the Golgi apparatus where it is packaged into vesicles for delivery to the cell surface for further processing by α-, β-, and γ-secretases following the non-amyloidogenic (constitutive) or amyloidogenic pathway (Fig. 3) (O'Brien and Wong, 2011; Ramaker et al., 2016). The non-amyloidogenic pathway leads to the production of non-pathogenic fragments, while the amyloidogenic pathway promotes the generation of Aβ peptides. Briefly, following the former pathway, APP is first cleaved by α-secretase within the Aβ sequence, thereby blocking Aβ production, to generate two proteolytic fragments: soluble APPα, and the corresponding C-terminal fragments, α-CTF/C83 (a protein stub that remains secured to the plasma membrane for further proteolytic processing) (Gandy et al., 1994; Roychaudhuri et al., 2009). Soluble APPα is recycled back to the cell surface by the recycling compartments or delivered to the lysosome for degradation through the endosomal–lysosomal system (Caster and Kahn, 2013; Golde et al., 1992). In the amyloidogenic pathway, APP is cleaved by β-secretase, the major secretase in the brain, at the N-terminus of the Aβ sequence, thus generating soluble APPβ, and the corresponding C-terminal fragment, β-CTF/C99 (a membrane-associated fragment comprising the entire Aβ sequence). Both C99 and C83 are subsequently cleaved by γ-secretase within the transmembrane domain, resulting in the release of a nontoxic p3 fragment, APP intracellular domain, and Aβ peptide species of slightly different lengths (Fig. 3) (Cole and Vassar, 2007; Jarrett et al., 1993).

Secretase cleavage gives rise to an admixture of Aβ peptides composed of 39–43 amino acids, with Aβ40 (90%) and Aβ42 (10%) being the two major Aβ species (Gouras et al., 2000; Takahashi et al., 2002, 2013). Although the role of Aβ40 remains unclear, evidence suggests a role in modulating synaptic function (Puzzo et al., 2008; Russell et al., 2012). Aβ42, on the other

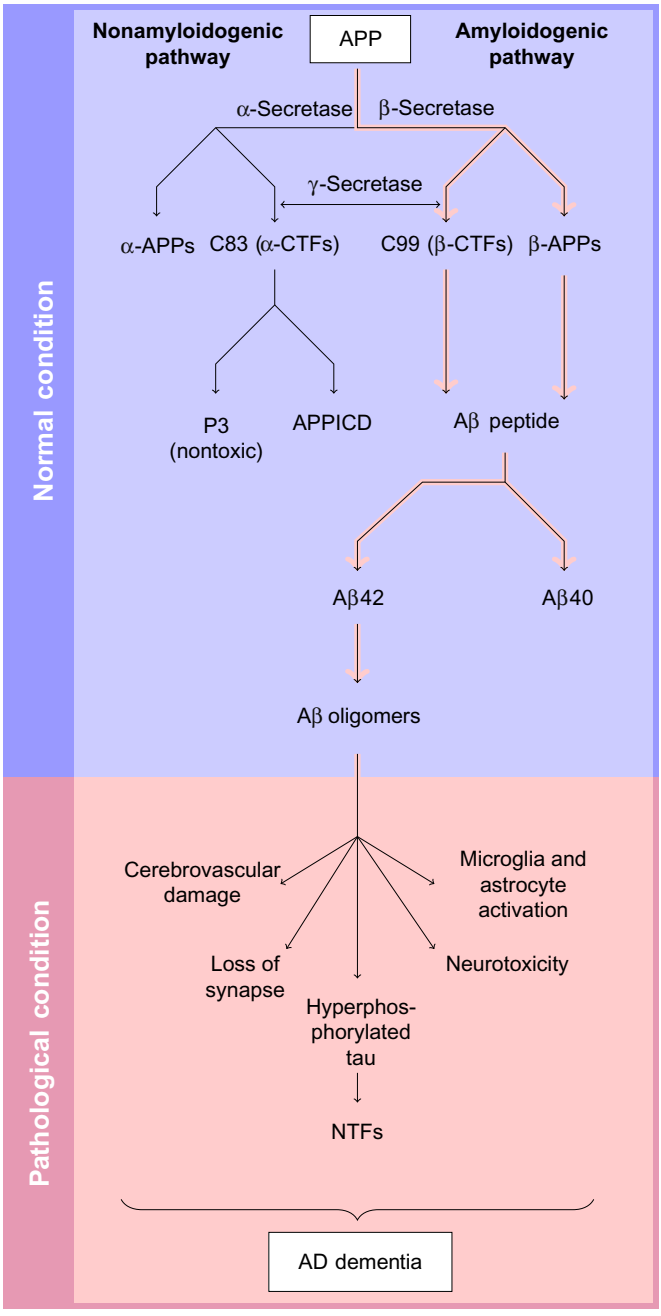


Fig. 3 The nonamyloidogenic and amyloidogenic pathway. Enhanced amyloidogenic pathway activity increases neuronal synthesis of aggregate prone toxic Aβ oligomers, which in turn leads to autophagy and mitochondrial dysfunction as well as tubulin disruption, thereby driving neurofibrillary tangle (NFT) formation.

hand, has a higher tendency to self-aggregate and form higher order structures, including toxic Aβ dimers, trimers, and oligomers. These higher structures are able to coalesce to form fibrils in insoluble beta-sheet conformation that eventually deposit into diffuse senile plaques (Burdick et al., 1992; Gravina et al., 1995). Although autophagy is responsible for the bulk degradation of aberrant proteins, not all aggregate-prone proteins are fully amenable to autophagic degradation (Wong et al., 2008). For example, expression of human Aβ40 and Aβ42 in *Drosophila* brain has been shown to have differential effects on neuronal autophagic degradation (Ling et al., 2009). Studies have shown that although autophagy sequesters Aβ42, this aggregate-prone peptide in turn may decrease the degradative capacity of autophagy (Ling and Salvaterra, 2011; Ling et al., 2014). This was demonstrated by highly concentrated intracellular Aβ identified in autophagic vacuoles (AVs), which accumulate in affected neurons, especially with advancing age (Ling and Salvaterra, 2011). In contrast, sequestration of Aβ40 does not produce any detectible changes in either the neuronal autophagy activity or neurological defects in vivo, which is consistent with the ACH for AD pathogenesis (Hardy and Higgins, 1992).

Prior to senile plaque deposition, Aβ42 oligomers are able to induce oxidative damage, promote tau hyperphosphorylation, and lead to synaptic and mitochondria toxicity (Kaminsky et al., 2015; Lustbader et al., 2004). Moreover, during late disease progression, Aβ42 senile plaques have been found to activate microglia (Rosenmann, 2013). Microglial activation results in the production and release of proinflammatory cytokines, including IL-1β, TNF-α, and IFNγ, which in turn stimulate the nearby astrocytes to further exacerbate Aβ42 production and dispersal (Dal Prà et al., 2015). To this end, immunohistochemical analysis has revealed significantly higher Aβ42 levels in AD brains than control brains (Funato et al., 1998). Additionally, the extent of Aβ42 deposition is much greater in AD brains with disease progression, while Aβ40 shows little or no apparent age-dependent accumulation (Funato et al., 1998). It is well established that AD-causing mutations in APP and in presenilin 1 (PSEN1) and presenilin 2 (PSEN2) alter APP proteolytic processing in a manner that alleviates the relative levels of the Aβ42 peptides (Borchelt et al., 1996; Scheuner et al., 1996). Mutations in APP that lie within the Aβ sequence increase the self-aggregation of the resultant peptides, not their production, while mutations in PSEN1 and PSEN2 increase the relative production of the longer, more hydrophobic, and self-aggregating Aβ42 peptides (Kim and Kim, 2008; Weggen and Beher, 2012). Furthermore, the inactivation of PSEN1 and PSEN2 has been

shown to completely prevent Aβ generation (Herreman et al., 2000; Zhang et al., 2000). Although Aβ42 is generated at a 10-fold lower rate than Aβ40, the former peptide has consistently been shown to be the main component of Aβ plaques in AD (Iwatsubo et al., 1994).

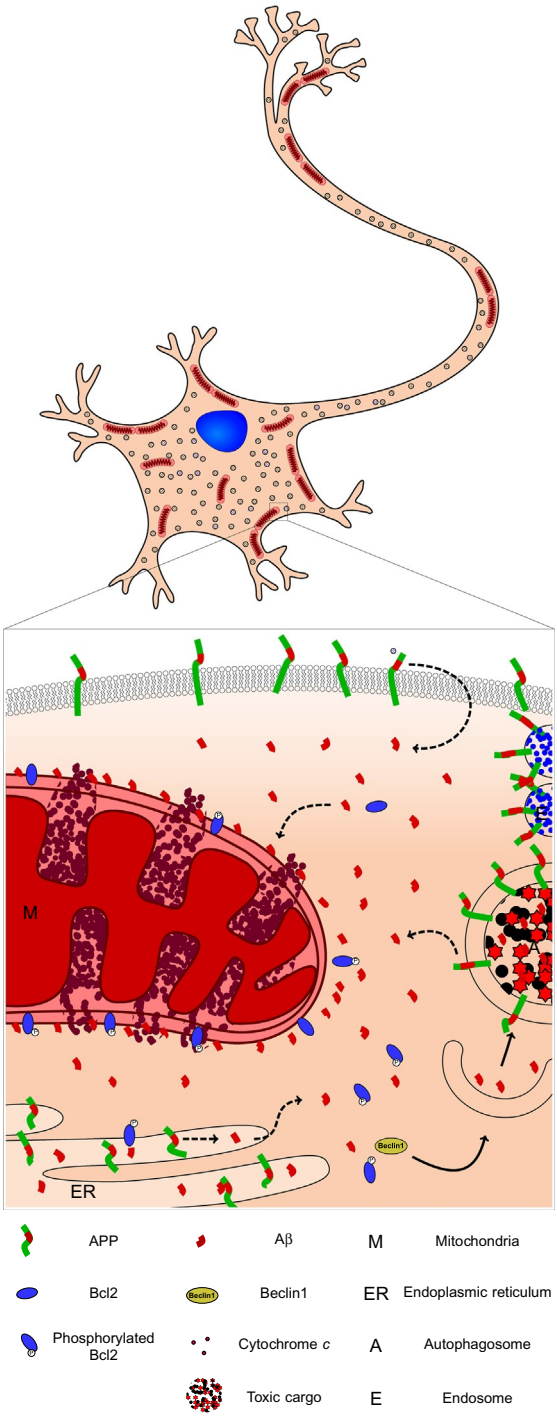
Originally, the ACH was mostly driven by genetic studies indicating the vast majority of early-onset familial AD mutations to confer a similar biochemical phenotype, i.e., an increased ratio of cerebral Aβ42, either through an increased Aβ42 production or decreased Aβ40 production, or a combination of both (Cavallucci et al., 2012; Cruts and Van Broeckhoven, 1998). And although the ACH takes a central position in AD-related research, the prevailing hypothesis does not entirely account for the complex pathophysiology of AD. Instead it seems that the role of Aβ in synaptic degeneration may act in concert with several other factors that impair the integrity of neuronal functions (Anand et al., 2014; Dal Prà et al., 2015). Growing evidence supports that dysregulated production of both Aβ and tau may synergistically disrupt synaptic activity and mitochondrial function, resulting in AD (Chételat, 2013; Musiek and Holtzman, 2015; Quintanilla et al., 2012; Teplow, 2013). Although many factors contribute to AD pathogenesis, imbalance in Aβ production and clearance has emerged as the most extensively validated and compelling therapeutic target for both genetic and sporadic AD, as both forms of the disease can be ascribed similar etiologies (Selkoe, 2012; Selkoe and Hardy, 2016).

4. AUTOPHAGY DYNAMICS AND AD PATHOGENESIS

In vivo and in vitro studies, using GFP-LC3, indicate that autophagosomes are very scarce in healthy neurons under nutrient-rich conditions (Mizushima et al., 2004; Nixon et al., 2000). Hence, it was originally proposed that basal autophagy occurs at very low levels in the normal brain (Mizushima et al., 2004). However, a growing body of evidence suggests that autophagosomes are not accumulating in healthy neurons due to the efficiency of the autophagic machinery, keeping the pool size at very low levels (Boland et al., 2008; Bordi et al., 2016; Nixon et al., 2005). It is now becoming increasingly clear that constitutive autophagy plays a critical role in neuronal proteostasis, with a degree of compartmentalization between soma and neuronal processes (Maday and Holzbaur, 2016). To what extend neurons indeed respond to a starvation stimulus remains conflicting (Alirezaei et al., 2010; Maday and Holzbaur, 2016). Inhibition of lysosomal degradation under nutrient-rich conditions leads to the robust

and rapid accumulation of autophagosomes in primary cortical neurons, suggesting a relatively high rate of autophagy in neurons (Boland et al., 2008; Lee et al., 2011a). Although studies as early as 1967 revealed the presence of abundant subcellular vesicles in the dystrophic neurites from postmortem AD brains (Suzuki and Terry, 1967), it was only much later through the application of electron microscopy that the first direct evidence of accumulated double membrane vesicles was clearly identified as AVs (Nixon et al., 2005). An extensive increase in AVs is notably abundant in dystrophic neuritic processes and synapses. The dystrophic neurites associated with Aβ plaques were enriched in autophagosomes and multivesicular and multilamellar bodies (Nixon et al., 2005, 2008). Since then, multiple studies have demonstrated a crucial role of autophagy in the development of AD due to its involvement in the production and deposition of Aβ, formation of NFTs and cell death (Caccamo et al., 2010; Lee et al., 2010).

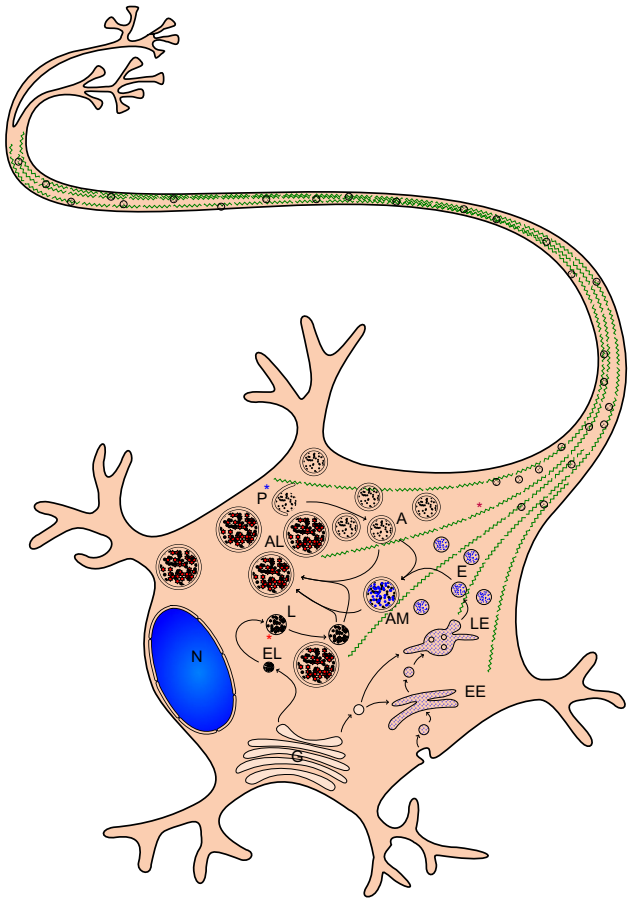
AV buildup may be due to elevated autophagic induction, impaired autophagosome clearance, or to a decrease in the rate of autophagosome formation combined with insufficient lysosomal fusion (Barnett and Brewer, 2011). Sanchez-Varo et al. (2012) demonstrated that the accumulation of AVs in the dystrophic neurites correlated with the appearance of AD pathology in an AD mouse model. Impaired autophagy at the level of induction or autophagosome formation points toward Beclin1, which has been shown to be reduced in the brains of patients in early- to late stages of AD (Lucin et al., 2013; Pickford et al., 2008; Rohn et al., 2011). Decreased Beclin1 expression is thought to be due to increased caspase 3-mediated cleavage thereof (Rohn et al., 2011). Interestingly, Beclin1 expression was found to be significantly reduced in brain regions most vulnerable to AD pathology, i.e., the entorhinal cortex and hippocampus (Pickford et al., 2008). This reduction in Beclin1 expression was further pronounced with AD progression, from mild cognitive impairment to severe AD (Fig. 4). These findings were confirmed in a follow-up study by Jaeger et al. (2010). Moreover, they observed that the expression of VPS34 was also equally reduced as Beclin1, and the level of LC3-II was significantly increased (Jaeger et al., 2010), which is in agreement with observations indicating that there is an accumulation of autophagosomes in AD brains (Nixon et al., 2000, 2005). In contrast, a genome-wide study found autophagy to be upregulated in the brains of patients with AD due to transcriptional upregulation of positive regulators of autophagy, as well as reactive oxygen species (ROS)-dependent activation of type III PI3 kinase, which is critical for the initiation of autophagy (Lipinski et al., 2010a,b).


M
ER
Beclin1
APP
Aβ
M
Mitochondria
Bcl2
Beclin1
ER
Endoplasmic reticulum
Phosphorylated Bcl2
Cytochrome c
A
Autophagosome
Toxic cargo
E
Endosome

Apart from autophagic dysfunction in the earlier stages of the pathway, mounting evidence suggests a lysosomal proteolytic failure. This is indicated by the accumulation of intermediate autophagy structures, such as autophagosomes, autolysosomes, or AVs in the AD brain (Nixon et al., 2005). Accumulating AVs were similar in morphology compared to those that result from blocking lysosomal proteolysis, deleting specific cathepsins, or that result from addition of lysosomal inhibitors (Boland et al., 2008; Koike et al., 2000). Autophagosomes contain abundant membrane-bound presinillin1 protein (PS1) and mutations associated to PSEN1s result in early-onset familial AD (Tan et al., 2014). As mentioned, PS1 is necessary for processing APP into Aβ (Martinez-Vicente, 2015), thus a mutation in PSEN1 results in increased production of Aβ peptide and subsequently the formation of Aβ plaques (Funderburk et al., 2010; Xia, 2000; Xia et al., 1998). Apart from playing a role in the γ-secretase complex, PS1 has other independent roles, including in lysosomal acidification as well as in facilitating autophagosome–lysosomal fusion (Esselens et al., 2004; Lee et al., 2010; Neely et al., 2011). Studies of fibroblasts derived from AD patients with a PSEN1 mutation have demonstrated a loss in lysosomal acidification and subsequent proteolysis leading to an accumulation of AVs (Lee et al., 2010).

Earlier findings suggest that the autophagic defect may result from a blockage of vesicle fusion among autophagosomes, endosomes, and lysosomes, which may impede the ability of these vesicles to acquire the necessary lysosomal enzymes required for cargo digestion (Fig. 5) (Boland et al., 2008; Nixon et al., 2005; Yu et al., 2005). Consistent with this, disrupted lysosomal proteolysis may account for defective autophagic degradation (Lee et al., 2011b; Wolfe et al., 2013; Xie et al., 2015). Incubation of cultured neurons with a cytotoxic concentration of Aβ has been shown to induce robust-free radical generation within the lysosomes, thereby disrupting lysosomal membrane proton gradient and inducing apoptosis

Fig. 4 Dysfunctional neuronal autophagy associated with AD. Neuronal function is compromised due to dysfunctional and fragmented mitochondria. ATP demands cannot be met. Autophagy pathology contributes not only to an increased autophagosomal, lysosomal, and endosomal pool size but also to enhanced production of Aβ in autophagosomal, endosomal, and endoplasmic reticulum membrane space. Increased Aβ toxicity decreases mitochondrial and autophagy function, enhancing cytochrome *c* release and favoring the buildup of toxic protein cargo. Reduced protein levels of Beclin1 offset the Beclin1–Bcl2 interactome, thereby disrupting autophagy and additionally sensitizing apoptosis onset.



P	Phagophore	EL	Early lysosome	AM	Amphisome
A	Autophagosome	E	Endosome	G	Golgi apparatus
AL	Autophagolysosome	EE	Early endosome	N	Nucleus
L	Lysosome	LE	Late endosome	*	Sites of therapeutic intervention
			Toxic cargo		

Fig. 5 Dysfunctional neuronal autophagy associated with AD. Enhanced endosome pathway activity, decreased lysosomal cathepsin enzyme activity, dysfunctional autophagosome maturation, autophagosomal/lysosomal fusion, as well as disrupted tubulin-mediated vesicle transport are defined molecular key events in the pathogenesis of AD, cumulatively favoring toxic protein aggregation. Distinct therapeutic targets exist aimed at preserving autophagy function or minimizing AD-associated autophagy pathology.

(Ditaranto et al., 2001). Moreover, endosomal sorting defects related to decreased levels of PI3P, required for proper functioning of retromer and other sorting regulators, have been linked to AD (Morel et al., 2013) and shown to favor the production of excessive levels of Aβ which may compromise the physical integrity/permeability of endolysosomal–autophagic compartments (Ditaranto et al., 2001; Friedrich et al., 2010; Ling et al., 2009; Umeda et al., 2011; Yang et al., 1998). In vivo overexpression of Aβ has also been found to lead to the progressive impairment of the endolysosomal–autophagic degradative capacity, resulting in a buildup of increasingly dysfunctional AVs (Ling et al., 2009). Although AVs appear protective and contribute to Aβ42 elimination during early stages, toxic Aβ load seems to increase with disease progression, resulting in impaired degradation due to the leakage of lysosomal proteins from abnormal AVs (Ling et al., 2009). The retrograde axonal transport may also compromise the endolysosomal–autophagic pathways in AD (Lee et al., 2011b; Muresan and Muresan, 2009; Perlson et al., 2010; Vicario-Orri et al., 2015). Under normal physiological conditions, immature AVs are transported retrogradely toward the soma for lysosomal degradation; however, in the AD brain, there is a significant buildup of AVs in dystrophic neurites, which is thought to be, in part, as a result of impaired transport. Indeed, pathological examination in AD brains has reported abnormal axonal transport in both early- and late AD progression (Bell and Claudio Cuello, 2006). This is further supported by the marked accumulation of AVs following inhibition of autophagosome delivery to lysosomes in a recent study by Boland et al. (2008). Moreover, phosphorylated tau has been shown to affect axonal transport and degradation (Rodríguez-Martín et al., 2013). The exact molecular defects underlying axonal transport failure, however, remain unclear. Taken together, intracellular Aβ accumulation may be both a cause and a consequence of an endolysosomal–autophagic dysfunction in AD.

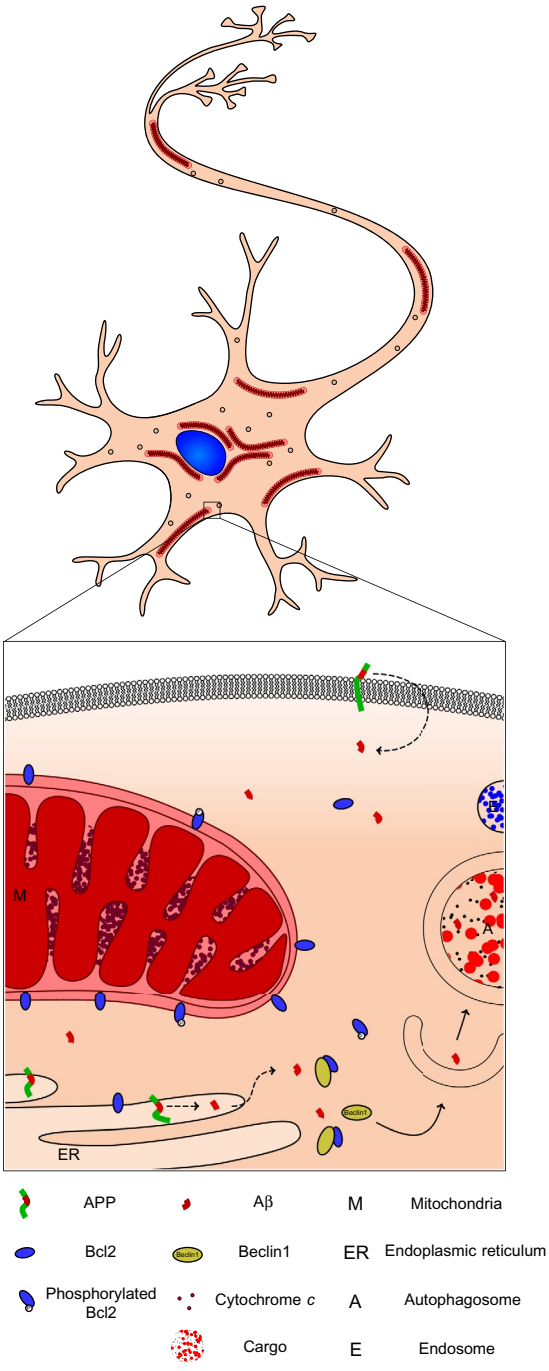
5. APP AND THE AUTOPHAGOSOME: SITE OF CLEARANCE AND SITE OF PRODUCTION

Although Aβ is normally distributed in the neuronal cytosol, emerging evidence suggests that a large fraction of Aβ may reside within different organelles, such as the mitochondria-associated membranes (Del Prete et al., 2017), dependent on the presence of APP, β-, and γ-secretase (Gouras et al., 2000; Nixon, 2004). These organelles include the ER, Golgi apparatus, endosomes, lysosomes (Koo and Squazzo, 1994; Yu et al., 2004),

and autophagosomes following autophagy activation (Mizushima, 2005). In vitro assays further confirm that γ-secretase can actively process sAPPβ and β-CTFs to produce Aβ inside autophagosomes, suggesting that these AVs may serve as a significant source of intracellular Aβ production in AD (Figs. 4 and 6) (Ohta et al., 2010; Yu et al., 2004, 2005). Several studies have shown that the inhibitors of lysosomal enzymes and lysosomotropic agents inhibit the processing of APP within acidic compartments, i.e., in the late endosomes, lysosomes, or autolysosomes (Caporaso et al., 1992; Cole et al., 1989). Given the well-characterized autophagic flux impairment in AD neurons (Nixon et al., 2000, 2005), studies have shown that toxic Aβ is generated in AVs and accumulated in neurons at the very early stage of AD, as well as in the transgenic PSEN1/APP mice (Yu et al., 2004, 2005). Immunolabeling indicated that AVs contain APP, Aβ, as well as high levels of Presinillin1 protein (Yu et al., 2005). The production of Aβ was increased with the induction of autophagy, whereas the suppression of autophagy clearly reduced Aβ load (Yu et al., 2005). Different types of cellular stress, such as hypoxia, ER stress, and nutrient deprivation, have also been shown to induce autophagic processing of APP (Dohi et al., 2012; Nijholt et al., 2011; Son et al., 2012b; Young et al., 2009). Findings from AD mouse models suggest that this stress-induced autophagy may remove the accumulated intracellular APP or Aβ, and in this way ameliorate cognitive deficits (Yang et al., 2011a,b,c). Moreover, studies have shown that intralysosomal accumulation of Aβ induced by oxidative stress can be prevented using an autophagy inhibitor, 3-methyladenine, in neuroblastoma cells (Zheng et al., 2011). Taken together, these studies suggest that neuronal stress conditions can alter the rate of APP processing. Autophagy seems to have an important role in APP processing, and the generation and clearance of Aβ (Fig. 4). Therefore, autophagy may be a variable therapeutic target in the prevention of Aβ accumulation (Hung et al., 2009). Although mechanisms underlying AD pathogenesis are complex and multifactorial, defects in autophagy pathway is most likely to precede the formation of Aβ plaques or NFTs (Correia et al., 2015). However, whether dysfunction of autophagy is the cause or result of AD remains unclear.

6. Aβ CLEARANCE AND THE ROLE OF AUTOPHAGY IN Aβ HOMEOSTASIS

Removal of Aβ from the brain is mediated by several pathways including proteolytic degradation by the proteases neprilysin and insulin degrading



enzyme, uptake by astrocytes and microglia, passive flow into the cerebrospinal fluid, and sequestration into the vascular compartment by a soluble form of the low-density lipoprotein receptor-related protein 1 (LRP1) (Deane et al., 2009). In the interstitial fluid of the normal brain, Aβ concentration is rigorously regulated by its rate of production from APP cleavage. The influx of Aβ into the brain across the blood–brain barrier (BBB) is mainly mediated by the receptor for advanced glycation end products (RAGE) and LRP1 (Deane et al., 2009; Shibata et al., 2000). Brain endothelial expression of RAGE has been found to be increased in both AD mouse models and AD patients (Deane et al., 2003; Miller et al., 2008; Yan et al., 1996), whereas LRP1 expression is reduced at the BBB (Deane et al., 2004; Donahue et al., 2006; Shibata et al., 2000). In normal brain plasma, a soluble form of LRP1 (sLRP1) sequesters approximately 70%–90% of plasma Aβ peptides, but in AD the levels of sLRP1 and its capacity to bind Aβ are reduced, thereby increasing free Aβ fraction in plasma (Deane et al., 2003). This in turn may lead to Aβ accumulation in brain, and the gradual oligomerization of Aβ peptides (Kayed et al., 2003; Lesné et al., 2006; Walsh et al., 2005).

Proteolytic degradation is thought to be a major contributor in preventing Aβ aggregation or deposition into extracellular plaques (Baranello et al., 2015). To this end, APP that does not get processed by the β-secretase is internalized into acidic endosomal compartments where mature and proenzyme forms of cathepsin B and D (candidate β-secretase) have been shown to cleave this protein in the endosome to generate β-CTF, which is then further processed to Aβ by γ-secretase within the endosome (Cataldo et al., 1997). Although a large fraction of the Aβ generated via the endolysosomal pathway is normally degraded by a number of proteases (extensively reviewed in Tanzi and Bertram, 2005), Aβ that escapes this pathway may be transported to the autophagy–lysosome pathway for degradation. Accordingly, dysfunction of these turnover mechanisms may lead to accumulation and aggregation of Aβ into extracellular plaques (Cataldo et al., 1997; Nixon et al., 2000). In support of this notion, induction of

Fig. 6 Neuronal function is characterized by high ATP demands that rely on efficient mitochondrial oxidative phosphorylation. High autophagy flux contributes not only to proteostasis and mitochondrial quality control but also maintains low levels of Aβ through autophagic clearance and the reduction of Aβ synthesis sites, including autophagosomes and endosomes. The functional Beclin1–Bcl2 interactome maintains both basal autophagy synthesis and high apoptosis thresholds.

autophagy has been shown to accelerate the clearance of both soluble Aβ and Aβ aggregates (Caccamo et al., 2010).

Intracellular Aβ may be sequestered by the autophagy machinery along with damaged organelles where Aβ is generated (Figs. 4 and 6) (Nixon, 2007; Yu et al., 2005). However, oxidative stress-induced autophagy has been found to increase Aβ generation (Zheng et al., 2011). Furthermore, induction of autophagy by rapamycin has been shown to lower intracellular Aβ levels and improve cognition (Caccamo et al., 2010), and long-term rapamycin treatment reduces Aβ plaque load in AD mouse models (Majumder et al., 2011). Several lines of evidence have also implicated autophagy in Aβ metabolism (Miners et al., 2008). First, under physiological conditions or during aging, AVs have been shown to be Aβ production sites (Mizushima, 2005; Yu et al., 2005). Second, cathepsin D and E (intracellular aspartyl proteases) have been found to influence Aβ peptide generation within the autophagy machinery as they exhibit β- and γ-secretase-like activity (Bagnoli et al., 2002). Accordingly, autophagy has been shown to facilitate the degradation and clearance of APP, as well as APP-CTFs and Aβ (Cho et al., 2014; Tian et al., 2014; Zhou et al., 2011).

In contrast, inhibition of cathepsin activity has been shown to result in a rapid and pronounced buildup of APP fragments (Bahr et al., 1994). The deletion of cathepsin B, a lysosomal cysteine protease that primarily degrades the aggregation-prone Aβ42 species, results in increased Aβ42 concentration and robust Aβ plaque deposition in an APP mouse model (Mueller-Steiner et al., 2006), whereas virus-mediated overexpression of this enzyme has the opposite effect (Ditaranto et al., 2001; Nixon, 2007). Autophagic-related proteins, Atg5, Atg12, and LC3, have also been found to associate with Aβ plaque and tangle pathologies in AD neuronal and endothelial cells (Ma et al., 2010). Consistent with this, recent work revealed an age-dependent downregulation of ATG1, ATG8a, and ATG18 expression in *Drosophila*, with a subsequent decrease in autophagic activity and increased Aβ generation (Omata et al., 2014). In another study, measurement of extracellular Aβ in autophagy-deficient mice revealed a 90% reduction in Aβ secretion, whereas restoration of autophagy enhanced Aβ secretion to normal levels (Nilsson and Saido, 2014; Nilsson et al., 2015), thus supporting the role of autophagy in Aβ metabolism. Endocytosis of exogenous Aβ, on the other hand, has been shown to inhibit autophagy by increasing mTOR signaling (Caccamo et al., 2010; Lafay-Chebassier et al., 2005), suggesting that Aβ may in turn also regulate autophagy despite being an autophagy substrate. Indeed, intracellular Aβ has been show to modulate

autophagy via the Akt-dependent pathway, RAGE-Ca^{2+}/CaM-dependent protein kinase β (CaMKKβ)-AMPK signaling, or induction of mitochondrial ROS generation (Lipinski et al., 2010a,b; Son et al., 2012a). Aβ may in this way create a feedback loop to promote its own degradation, thus constituting an intrinsic checkpoint for its own homeostasis (Hung et al., 2009). Together, these findings firmly support the essential role of autophagy in the production and clearance of APP and its cleavage products. However, it also highlights the complexity of its dual role both in removal and production of Aβ.

7. THE PROTEIN SIGNATURE OF AUTOPHAGIC FAILURE IN AD

Presymptomatic stages of AD occur at least one decade before the clinical onset of AD (Bateman et al., 2012; Sonnen et al., 2008); therefore, specific protein biomarkers are needed to both monitor AD progression and reflect early stage pathogenic events. Protein signatures, such as elevated expression levels of specific aggregation-prone proteins and suboptimal levels of protein homeostasis components, in healthy human brains may serve as early prediction to AD vulnerability (Freer et al., 2016). In the AD brain, prefrontal cortex-related neuritic plaque and NFTs start to appear around Braak stage III (Braak and Braak, 1991; Braak et al., 2006). This region therefore reflects changes in gene expression before, during, and after the onset of AD and has been used to investigate Braak stage-related neuropathology (Bossers et al., 2010). During Braak stages II–III, right at the onset of plaque and fibril formation in the prefrontal cortex, gene expression patterns show the most variation. Intracellular Aβ abundance increased during Braak stages I–III, followed by a clear decrease during stages IV–VI. Very early on in the disease, during the presymptomatic phase, increased synaptic activity and changes in plasticity is observed. Related gene expression decreases again during the later Braak stages leading to reduced synaptic activity resulting from plaque and neurofibrillary pathology. These changes likely contribute toward the mild cognitive impairments observed during these stages (Bossers et al., 2010; Belbin et al., 2007; Craft et al., 1998).

The lysosomal network also reflects some of the earliest changes in AD (Ihara et al., 2012). Early pathological events include neuronal endosomal enlargement and the upregulation of genes linked to endocytosis-related proteins (Bronfman et al., 2007; Ginsberg et al., 2010; Jiang et al., 2010). These early events are followed by increased lysosomal biogenesis,

impairment in autophagy and in genes and proteins related to the lysosomal network, suggesting failed lysosomal clearance (Boland and Nixon, 2006; Nixon et al., 2005; Yu et al., 2005). A recent study investigating a broad range of lysosomal network proteins reported a significant increase in six of these proteins in the cerebrospinal fluid from AD patients compared to healthy controls (Armstrong et al., 2014). Included were the early endosomal antigen 1 (EEA1), lysosomal-associated membrane proteins 1 and 2 (LAMP-1, LAMP-2), LC3, and Rab3 and Rab7 (Armstrong et al., 2014). The accumulation and storage of undigested substrates in the AD brain reflect a major defect in lysosomal clearance (Nixon et al., 2005). Growing evidence from AD mouse models suggests that restoration of lysosomal proteolytic function may ameliorate deficits (Lee et al., 2015; Yang et al., 2011a,b,c). Reports on lysosomal dysfunction in AD models are more widespread (Boland et al., 2008; Lee et al., 2010) and less conflicting than reports of autophagic induction and autophagosome formation (Lipinski et al., 2010a; Pickford et al., 2008; Sun et al., 2014; Tramutola et al., 2015). Data from a recent genome-wide screen indicated that autophagy-regulating gene expression patterns observed in normal aging are reflected in the AD brain (Lipinski et al., 2010b). The type III PI3 kinase pathway is essential for the upregulation of autophagy via Aβ, and it was recently demonstrated that ROS play a crucial role as mediator in this context (Lipinski et al., 2010a). Lysosomal blockage is also caused by Aβ, an event independent from ROS activity. It was reported that autophagy is transcriptionally downregulated in correlation with normal aging in the human brain; however, autophagy seems to be upregulated in the brains of AD patients, indicating possible compensatory mechanisms (Lipinski et al., 2010a).

In a recent study, variations in autophagy within the vulnerable CA1 hippocampal neuronal population of the AD brain, with a specific focus on early- vs late-stage variations in the major steps of the autophagic pathway (initiation and flux), were investigated (Alldred et al., 2012; Bordi et al., 2016; Ginsberg et al., 2010, 2012). A prominent upregulation in autophagy-related genes within this region reflected increases in both autophagosome formation and lysosomal biogenesis in the early stages of AD (Bordi et al., 2016). Gene and protein expression levels for autophagosome components and increased LC3-positive puncta further indicated increased hippocampal autophagosome formation. Autophagic substrate targeting and sequestration seem to be competent and upregulated in AD (Bordi et al., 2016), most likely triggered by increased accumulation of toxic Aβ and tau. Furthermore, autophagosome–lysosomal fusion

remains intact in the hippocampus during early stage AD (Bordi et al., 2016). Despite increased autophagosomal and lysosomal formation, an impaired ability to clear autophagic substrates persists, impeding autophagic flux. This was reflected by autolysosomal accumulation of LC3-II and SQSTM1/p62, together with an increase in autolysosomal size and total area. These findings provide plausible basis for the extreme autophagic pathology and the massive accumulations of substrate-ridden autolysosomes within dystrophic neurites in AD as previously described (Serrano-Pozo et al., 2011).

8. AUTOPHAGY CONTROL: THERAPEUTIC INTERVENTIONS IN AD

Given the extensive evidence supporting a crucial role for autophagy in neuropathology summarized earlier, there has been a growing interest in manipulating this pathway as a potential therapeutic target for AD (Table 1). Multiple studies have provided evidence that autophagy induction, using pharmacological agents or caloric restriction, reduces aggregate prone proteins and associated disease pathology in models of AD. Indeed, in an APP transgenic mice model, long-term inhibition of mTOR by rapamycin alleviated AD cognitive like deficit and lowered toxic Aβ42 levels (Spilman et al., 2010). Use of rapamycin in a mouse model of AD rescued cognitive deficits and ameliorated tau pathology by increasing autophagy (Caccamo et al., 2010. In another study using 3xTg-AD mice, they reported a reduction in amyloid plaques, NTFs, and cognitive defects upon autophagy induction with rapamycin (Majumder et al., 2011). In addition, in APP transgenic mice, the use of a lentiviral vector expressing Beclin1 reduced intracellular and extracellular amyloid pathology (Pickford et al., 2008). Rapamycin analog CCI-779 has also been shown to have neuroprotective roles in AD. In APP/PS1 mice CCI-779 administration reduced Aβ plaques and alleviated learning and memory deficits (Jiang et al., 2014a), while it reduced levels of hyperphosphorylated tau and rescued learning deficits in P301S tau transgenic mice (Jiang et al., 2014b) by autophagy induction. Despite the promising effects of rapamycin and its analogs, in inducing autophagy, these compounds cannot be used for a longer term as required in chronic diseases like AD as they afford protective roles by inhibiting mTOR activity which is essential in protein synthesis.

Other pharmacological agents that upregulate autophagy induction independent of mTOR such as lithium, trehalose, SMER-28, methylene B, and carbamazepine have been used successfully in models of AD

Table 1 Autophagy Modulation in Experimental Models of AD

Drug Intervention	Model	Target	Results	References
Rapamycin	APP Tg mice	Induction	Alleviated cognitive-like deficit and lowered the Aβ42 levels	Spilman et al. (2010)
Rapamycin	3xTg-AD mice (Tg human/APPswe/Tg human tau P301L)	Induction	Rescued cognitive deficits and ameliorated tau pathology	Caccamo et al. (2010)
Rapamycin	3xTg-AD mice	Induction	Reduction in amyloid plaques, NTFs, and cognitive defects	Majumder et al. (2011)
Lentiviral vector expressing Beclin-1	APP Tg mice	Induction	Reduced intracellular and extracellular amyloid pathology	Pickford et al. (2008)
CCI-799	APP/PS1	Induction	Reduced Aβ plaques and alleviated learning and memory deficits	Jiang et al. (2014a)
CCI-799	P301S tau Tg mice	Induction	Reduced levels of hyperphosphorylated tau and rescued learning deficits	Jiang et al. (2014b)
Lithium	AβPPSWE/PS1A246E	Induction	Reduced amyloid β production, senile plaques formation, improved memory deficits	Zhang et al. (2011)
Lithium	Tg human tau P301L	Induction	Decreased soluble tau levels and NFTs	Shimada et al. (2012)
Trehalose	APPSWE	Induction	Reduced levels of amyloid β peptide and tau plaques	Perucho et al. (2012)
Methylene B	3xTg AD	Induction	Decreased soluble Aβ, no reduction in tau phosphorylation	Medina et al. (2011)
Sodium valproate, sodium butyrate	APP Swedish/PS1E9 mice	Axonal transport and maturation	Decrease memory deficit	Guan et al. (2009) and Kilgore et al. (2010)
Paclitaxel	Tau transgenic mice	Axonal transport and maturation	Ameliorate motor impairment and to improve fast axonal transport	Zhang et al. (2005)
Genetic ablation of cysteine B	TgCRND8 mice overexpessing human APP695	Lysosomal proteolysis	Reduce amyloid pathology and memory deficits	Yang et al. (2011a,b,c)
Genetic overexpression of cathepsins	hAPP/Cat B (+/–)	Lysosomal proteolysis	Reduced the abundance of Aβ1–42	Mueller-Steiner et al. (2006)

Distinct therapeutic targets exist aimed at preserving autophagy function by targeting the autophagy machinery (*blue*), the tubulin network/axonal transport system (*green*), or lysosomal proteolysis (*red*).

(Congdon et al., 2012; Li et al., 2013; Medina et al., 2011; Perucho et al., 2012; Schaeffer et al., 2012; Shimada et al., 2012; Tian et al., 2011; Zhang et al., 2011). For example, lithium reduced soluble tau level, NFTs, Aβ production, and alleviated memory deficits and motor disturbances in transgenic models of AD and in a model of tauopathy (Shimada et al., 2012; Zhang et al., 2011). Other pharmacological agents such as clonidine, rilmenidine, stimulate autophagy induction; however, they have not been used as a form of intervention in AD models yet. Although induction of autophagy seems to be beneficial in many instances, the timing of the intervention in the disease progression as well as point of dysfunction in the autophagic pathway is crucial when implementing autophagy as a therapeutic approach. Increased autophagic induction before the development of AD-like pathology in 3xTg mice reduced the levels of soluble Aβ and tau, whereas its induction after the plaques and tangle formation had no

effect on AD pathology or cognition (Majumder et al., 2011). In addition, if the autophagosome clearance is impaired, induction of autophagosome formation may be deleterious due to the harmful accumulation of AVs that are not being cleared efficiently (Liang and Jia, 2014). Yu et al. (2005) found that autophagosomes in AD brains may be a major reservoir of Aβ; therefore, enhancing formation of new autophagosome without the parallel increase in their degradation may result in increased Aβ production, accumulation, and subsequent toxicity (Nixon, 2007). Interventions aimed at restoring lysosomal proteolysis hold promise as a therapeutic target for AD. Indeed, enhancing lysosomal activity by genetic ablation of cysteine B has been shown to enhance the clearance of autophagic substrates and to reduce amyloid pathology and memory deficits in mouse models (Yang et al., 2011a,b, c). In addition, genetic overexpression of cathepsins reduced the abundance of Aβ42 through limited proteolysis, suggesting a possible therapeutic strategy for AD through activation of cathepsin B (Mueller-Steiner et al., 2006). Both these strategies demonstrate efficiency in restoring lysosomal activity in mouse models of AD (Sun et al., 2008; Yang et al., 2011a,b,c, 2014). To date, no pharmacological compounds of this nature have been developed.

Since autophagosome transport is also implicated in AD pathogenesis, microtubule stabilizers that aid transport have been identified. These include paclitaxel, epothilone D, and HDAC inhibitors such as sodium valproate, sodium butyrate, and spermidine. Sodium valproate and sodium butyrate have been found to decrease memory deficit in the APP Swedish/PS1ΔE9 mouse model of AD (Guan et al., 2009; Kilgore et al., 2010). Paclitaxel has been shown to ameliorate motor impairment and to improve fast axonal transport and morphology in tau transgenic mice (Zhang et al., 2005); however, there are also conflicting findings with regards to the role of paclitaxel in autophagy induction (Nunes et al., 2013; Xie et al., 2010). Paclitaxel potentially enhances autophagic transport and fusion; however, further investigations are required as the bioavailability of this drug in the brain is suboptimal. Given the complexity of the underlying mechanisms in AD pathogenesis, and the multiple steps that may be defective in the autophagic pathway, it is becoming increasingly clear that a multifaceted therapeutic approach may be required. It is necessary to design an approach that not only increases autophagic induction, but also aims to improve autophagosome transport together with lysosomal proteolysis. Designing such a combinatory approach may be challenging since the stage of progression and the cause of the disease will have to be taken into account. Taken together, it is clear that autophagy modulation holds promise in serving as a powerful therapeutic

intervention to attenuate AD pathogenesis and rescue cognitive impairment.

9. SUMMARY AND FUTURE DIRECTIONS

The role of autophagy dysfunction in the pathogenesis of AD raises many questions. Although autophagy dysfunction and pathology are known to contribute to proteotoxicity and neuronal apoptosis, the dynamic interplay between endocytosis, autophagic flux, and lysosomal proteolytic function is complex. This complexity is amplified by the molecular crosstalk between autophagy and apoptosis, as indicated by the Beclin1–Bcl2 interactome. Dealing with autophagic flux and cytochrome release thresholds underpins the importance of a dynamic approach in assessing this pathology. This may include single-cell analysis at large, where each cell may be treated as a system with distinct regions of functionality, such as regions of differential ATP demand and proteotoxicity. Neuronal cell death in AD is not a rapid event and requires an understanding of the history of the cell, the time spent in agony of autophagy failure and Aβ toxicity. Dissecting the relationship between autophagic flux, toxic proteinaceous cargo (in particular Aβ), and the dynamic changes in the magnitude of the site of synthesis and site of degradation will be an important challenge. New insights into the dynamic nature of vesicular trafficking, including ATP-dependent tubulin-mediated processes, autophagosomal, and endosomal pool sizes, lysosomal proteolytic function, and mitochondrial cytochrome *c* release thresholds raise interesting mechanisms for the control of neuronal fate. The relationship between vesicular pool sizes, protein cargo, and autophagic flux needs to be established in a highly quantitative manner, which will allow better identification of targets for drug design. The distinct and dynamic contribution of endosomal, autophagosomal, lysosomal, and tubulin dysfunction in the progression of AD pathology calls for a precision controlled and yet combinatory approach for therapeutic intervention. The identification of better peripheral markers that may report on these early events of neuronal autophagy dysfunction will be an important avenue for the future.

Acknowledgments

The authors wish to acknowledge financial support from the South African National Research Foundation (NRF), the Medical Research Council (MRC), as well as the Cancer Association of South Africa (CANSA).

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